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Original article

Diagnostic value of TCRγ rearrangement analysis by PCR in early mycosis fungoides: A clinicopathologic study

Hasna Hadiri[1], Wafa Mokni[2,3]*, Sarra Yacoub[2,3], Zeineb Nfikha[2,3], Safwen Frini[2,3], Badreddine Sriha[2,3], Moncef Mokni[2,3]

[1]*Department of Pathology- CHU Mohammed VI, Marrakech, Morocco*
[2]*Department of Pathology - CHU Farhat Hached, Sousse, Tunisia*
[3]*Research Laboratory LR12SP08- CHU Farhat Hached, Sousse, Tunisia*

Abstract

Background: Mycosis fungoides (MF) is the most common type of primary cutaneous T-cell lymphoma. Diagnosing early-stage MF remains challenging because its clinical and histopathological features frequently mimic benign inflammatory dermatoses. Molecular analysis of T-cell receptor (TCR) gene rearrangements has emerged as a valuable adjunctive tool in this context. The objective of this study was to evaluate the diagnostic value of polymerase chain reaction (PCR) detection of TCR gamma (TCRγ) gene rearrangements in patients with suspected early MF, and to determine its utility within an integrated clinicopathological approach.

Methods: This retrospective study included 16 patients who underwent skin biopsy for lesions suspicious for MF between 2019 and 2021 at the Farhat Hached University Hospital, Sousse. Clinical data, histopathological findings, immunohistochemical profiles, and molecular analyses were reviewed. T-cell clonality was assessed via PCR amplification of TCRγ gene rearrangements using formalin-fixed paraffin-embedded tissue.

Results: The study population comprised 16 patients aged 4 to 73 years (mean age: 43.5 years). Histopathological examination revealed superficial lymphoid infiltrates with varying degrees of epidermotropism, which were suggestive of but not definitive for MF. Immunohistochemical analysis showed a predominance of CD3-positive T lymphocytes with CD4 predominance in most cases. PCR analysis detected clonal TCRγ rearrangements in 8 cases (50%), polyclonal patterns in 7 cases, and an oligoclonal pattern in 1 case. After integrating clinical, histological, immunophenotypic, and molecular findings, the final diagnosis was MF in 13 patients and chronic inflammatory dermatosis in 3 patients.

Conclusion: PCR detection of TCRγ gene rearrangements represents a valuable ancillary technique in the diagnostic evaluation of suspected early MF. However, molecular results must always be interpreted in conjunction with clinical, histological, and immunophenotypic findings to avoid diagnostic pitfalls.

Keywords: Mycosis fungoides; Cutaneous T-cell lymphoma; TCRγ; PCR; Clonality analysis

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1. Introduction

Mycosis fungoides (MF) accounts for approximately 50% of all primary cutaneous T-cell lymphomas [1]. Identifying the disease at its inception is exceptionally difficult because early clinical features and histological changes closely mimic benign conditions. Consequently, early MF is frequently underdiagnosed, necessitating reliable ancillary diagnostic methods.

Molecular analysis of T-cell receptor (TCR) gene rearrangements, particularly the TCRγ genes, has emerged as a highly sensitive approach for identifying lympho-proliferative disorders. However, the specificity of TCRγ PCR remains a subject of discussion, as monoclonal rearrangements have also been observed in certain chronic inflammatory dermatoses. The present study aimed to evaluate the integration of molecular findings with clinicopathological and immunophenotypic data to define the precise role of TCRγ PCR in the diagnosis of early MF.

2. Materials and Methods

Inclusion criteria

Patients with skin lesions suggestive of MF who underwent skin biopsies showing signs of early-stage disease were eligible. Histological characteristics required included a superficial lymphoid infiltrate, focal epidermotropism, and mild lymphocytic atypia.

The immunohistochemical (IHC) profile had to be consistent with MF, specifically exhibiting a T-cell phenotype (predominance of CD3 and CD4) with partial loss (less than 10% of the lymphoid population) of other T-cell markers (CD2, CD5, or CD7). Between 2019 and 2021, 21 patients met these criteria.

Five patients were excluded: four due to insufficient tumor material, inadequate fixation (incorrect formalin dilution or suboptimal fixation time), or degraded DNA; and one who had received local or systemic treatment prior to the biopsy. Consequently, a total of 16 patients were enrolled.

* Correspondence: Dr. Wafa Mokni, wafa.mokni@gmail.com

Sample preparation and histopathology

Biopsies were fixed in formalin and embedded in paraffin (FFPE). Serial sections of 3–5 µm thickness were prepared from the paraffin blocks. One section was stained with hematoxylin and eosin (H&E) to assess the cellular architecture and lymphoid infiltrate, while the remaining sections were reserved for IHC and DNA extraction.

Immunohistochemical analysis

FFPE tissue sections were processed for IHC using a panel of specific anti-T lymphocyte antibodies: CD3, CD4, CD8, CD5, CD7, and anti-CD20 (a B-lymphocyte marker). This analysis confirmed the T-cell lineage of the infiltrate and allowed evaluation of the CD4/CD8 ratio. A profile consistent with early MF was defined as a CD3-positive T-cell infiltrate with a clear predominance of CD4.

Molecular analysis of TCRγ gene rearrangements

Following deparaffinization, genomic DNA was extracted from the paraffin sections using a commercial kit according to the manufacturer's instructions. DNA quality and concentration were verified before amplification. T-cell clonality was determined by PCR analysis of TCRγ gene rearrangements. Four oligonucleotides corresponding to the four Vγ segment families and four oligonucleotides corresponding to the Jγ junction segments were utilized in a multiplex PCR reaction (50 µL) performed in a thermal cycler. After 40 cycles, 30 µL of the amplified products were loaded onto a 6.5% polyacrylamide gel. PCR runs included positive, negative, and blank water controls to ensure accuracy and reproducibility.

Analysis of PCR product

All PCR products underwent gel electrophoresis. Monoclonality was defined as the detection of a single, distinct, homogeneous amplified fragment. Polyclonality was defined as the presence of multiple, heterogeneous fragments forming a smear or multiple weak bands. An oligoclonal profile was defined by the presence of two to five distinct bands of comparable intensity.

The molecular results were subsequently integrated and discussed in light of clinical, histological, and immunohistochemical data to establish the final diagnosis. Due to the small sample size, formal statistical analysis was not conducted.

3. Results

Clinical features

The study population consisted of 16 patients ranging in age from 4 to 73 years, with a mean age of 43.5 years. The cohort included 10 women and 6 men (female-to-male ratio of 5:3) (Table 1). Clinically, patients presented with persistent cutaneous lesions, including erythematous patches, plaques, or hypopigmented macules. These lesions were characteristically chronic and resistant to conventional topical dermatological treatments.

Table 1. Clinical presentation features of specimens in 16 patients correlated with histological findings

Histological Category	Suggestive MF (n=3)	Diagnostic MF (n=9)	Non diagnostic MF (n=4)
Average age	44	36	60
Sex (M: F)	2:1	4:5	0:4
Clinical findings	Hypopigmented or reddish plaques Reddish squamous patches	Hypo or hyperpigmented plaques Reddish patches and macules	Atrophic or reddish squamous plaques

Histopathologic findings

Histological examination revealed superficial dermal lymphoid infiltrates in all cases (Fig. 1a). Epidermotropism of lymphocytes was observed in several specimens, although it was generally mild. In some cases, the histological features were subtle and insufficient on their own to establish a definitive diagnosis of MF (Fig. 1a and 1b). Notably, four cases initially presented with features strongly resembling chronic inflammatory dermatoses, such as chronic eczema. Only three cases could be diagnosed firmly as early MF based solely on standard histological criteria.

Immunohistochemical findings

IHC analysis demonstrated that the infiltrate was predominantly composed of CD3-positive T lymphocytes, with most cases showing a marked predominance of CD4-positive cells. Only rare CD20-positive B lymphocytes were identified within the infiltrates (Fig. 1c and 1d). These findings supported a T-cell lymphoproliferative process compatible with MF.

Molecular findings

PCR analysis of TCRγ gene rearrangements revealed a clonal rearrangement in 8 cases (50%), a polyclonal pattern in 7 cases, and an oligoclonal pattern in 1 case (Fig. 2, Table 2).

Final diagnosis

After thorough correlation of clinical, histological, immunophenotypic, and molecular data, the final diagnoses were established as follows Mycosis fungoides in 13 cases, and chronic inflammatory dermatosis in 3 cases (Table III).

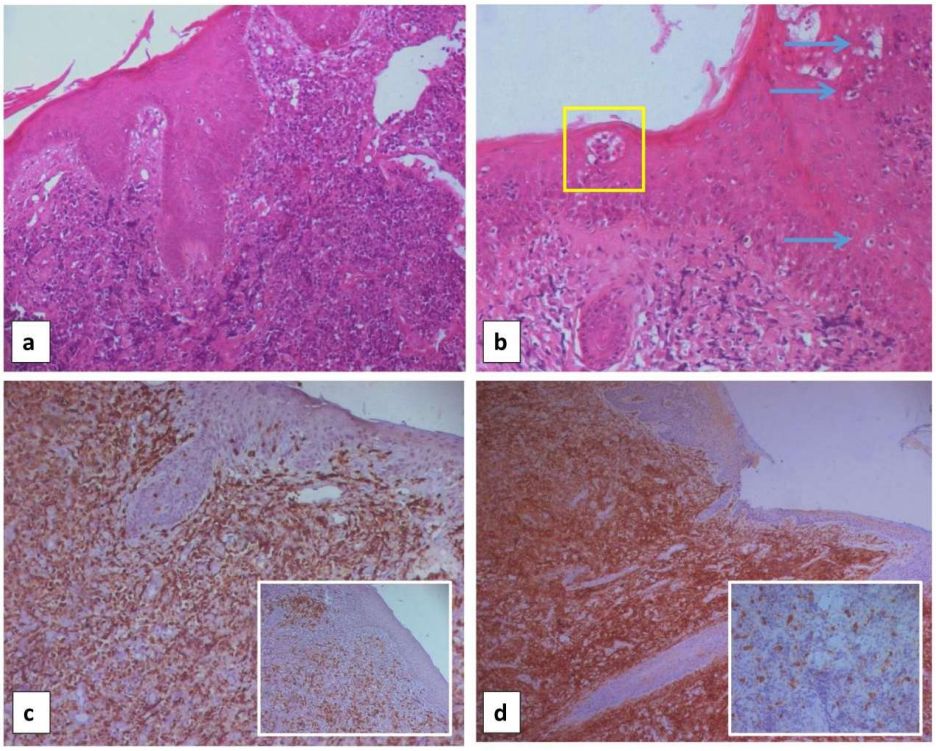

Fig. 1. Histopathological and immunohistochemical features of mycosis fungoides.

(a) Hematoxylin and eosin (H&E) staining at low magnification showing a superficial dermal lymphoid infiltrate with epidermal involvement. (H&E, x 100). (b) Higher magnification reveals epidermotropism of atypical lymphocytes (arrows) and a Pautrier microabscess (yellow box). (H&E, x 100). (c) Immunohistochemical staining demonstrates diffuse positivity of the infiltrating lymphoid cells for CD3; the inset shows a few CD20 lymphocytes, excluding a B-cell phenotype. (IHC, x100). (d) The neoplastic lymphoid cells show strong expression of CD4, while the inset highlights scarce CD8-positive cells, supporting a predominance of CD4-positive T lymphocytes. (IHC, x100 and x200).

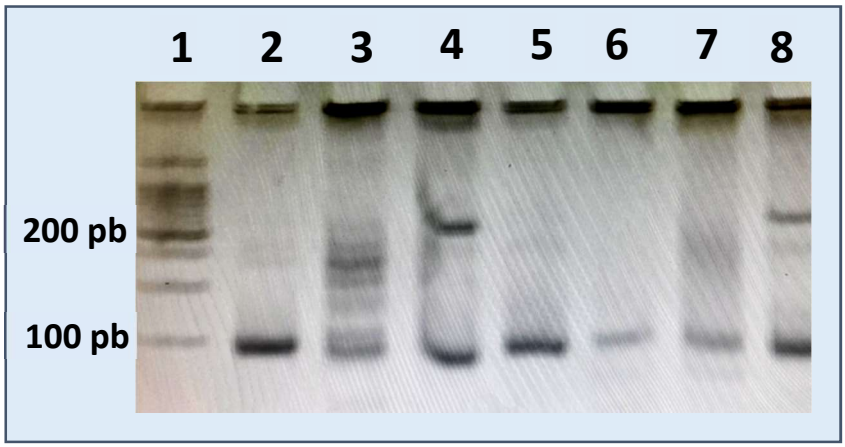

Fig. 2. Representative gel electrophoresis of PCR analysis.

Lane 1: Molecular weight marker; lane 2: Negative control (without DNA); lanes 3 and 4: Polyclonal profile; lanes 5, 6 and 7: monoclonal profile; lane 8: positive control.

Table 2. Frequency of PCR positivity according to lesions

Morphology	Number of lesions	Polymerase chain reaction positive
Patch	4	2
Plaque	9	6
Macule	3	1

Table 3. Frequency of PCR positivity according to histological diagnosis groups

Diagnosis	Number of patients	Polymerase chain reaction
Suggestive MF	3	3*
Diagnostic MF	9	6
Non-diagnostic MF	4	0
Total	16	9

* : 2 monoclonal and 1 oligoclonal

4. Discussion

Mycosis fungoides is the most common form of primary cutaneous T-cell lymphoma; however, diagnosing its early stages remains a major clinical hurdle because it heavily overlaps with the clinicopathological features of benign inflammatory dermatoses (BIDs) [1]. A definitive diagnosis should be established by combining clinicopathological findings with data from ancillary tests, rather than relying upon a single defining criterion. This comprehensive approach is highlighted by Miyagaki, who discusses an updated diagnostic algorithm displaying high sensitivity and specificity. The development of accurate, accessible diagnostic methods and the widespread dissemination of new technologies are necessary to improve the management of patients suspected of having early-stage MF, while exercising caution to avoid misdiagnosis or the inappropriate use of immunosuppressants [2].

Early MF lesions frequently display only subtle histological abnormalities, such as mild epidermotropism and limited cytologic atypia. Consequently, diagnosis routinely relies on a combination of clinical, histopathological, immunophenotypic, and molecular findings [3].

This integrated diagnostic strategy is formalized in the diagnostic algorithm proposed by the International Society for Cutaneous Lymphoma (ISCL), which incorporates the molecular detection of T-cell clonality as a key supportive criterion for early MF diagnosis [4, 5, 6].

In this context, PCR-based analysis of T-cell receptor (TCR) gene rearrangements serves as an important ancillary technique.

The principle of this method relies on identifying a clonal T-cell population derived from a single neoplastic precursor. Among the TCR loci, the TCRγ gene is the most frequently analyzed due to its relatively simple genomic organization and the technical feasibility of PCR amplification from FFPE tissues [7, 8].

In the present study, clonal TCRγ gene rearrangements were detected in 50% of cases. This detection rate is highly consistent with previously reported data for early MF, where the positivity rate of TCRγ clonality typically ranges from approximately 40% to 71% [9]. These variations are largely attributable to differences in study populations, methodological approaches, DNA extraction protocols, and the proportion of neoplastic cells present within the biopsy specimens [10].

The sensitivity of TCR clonality detection also varies according to the specific molecular techniques used. The BIOMED-2 multiplex PCR protocol, which allows simultaneous analysis of multiple TCR gene segments, has significantly improved clonality detection in lymphoid proliferations. Studies evaluating the BIOMED-2 approach have reported sensitivity rates of approximately 60–70% and specificities exceeding 80% for the diagnosis of cutaneous T-cell lymphomas [11].

Furthermore, when multiple TCR loci are analyzed simultaneously, the diagnostic yield increases substantially. In one large study using combined TCRγ and TCRβ assays, clonality was detected in up to 89% of cutaneous T-cell lymphoma cases, highlighting the clear benefit of evaluating multiple molecular targets [12].

More recent studies have also suggested that analysis of TCRβ rearrangements may provide higher sensitivity than TCRγ alone in early MF.

For example, a retrospective study evaluating 61 skin biopsies demonstrated that TCRβ PCR detected clonality in 83% of early MF cases, compared with only 43% using TCRγ PCR [13]. These findings indicate that a combined analysis of multiple TCR loci may improve diagnostic sensitivity, particularly in early disease stages where the tumor cell burden is low.

Despite these advantages, PCR-based clonality analysis has key limitations. Clonal T-cell populations may occasionally be detected in chronic inflammatory dermatoses, particularly in conditions characterized by persistent, antigen-driven T-cell stimulation. As a result, the presence of a clonal T-cell population does not automatically equate to malignancy [14]. Conversely, false-negative results can occur when the proportion of neoplastic cells in the biopsy specimen is very low or when the sample contains a dense, reactive background of benign T cells. Early MF lesions may therefore demonstrate polyclonal or oligoclonal patterns despite the presence of an underlying neoplastic T-cell clone [15].

For these reasons, molecular clonality testing should strictly be interpreted within the context of clinicopathological correlation [6]. In our series, the integration of clinical data, histological findings, immunophenotypic characteristics, and molecular results allowed for a definitive diagnosis of MF in the majority of cases.

Immunohistochemical analysis showing a predominance of CD4-positive T lymphocytes provided additional support for the diagnosis in several cases, although immunophenotypic alterations alone remain insufficient to establish malignancy.

The present study has several limitations, notably the relatively small sample size, which limits the statistical power of our analysis and may influence the observed detection rate of TCRγ clonality.

In addition, the retrospective design and the reliance on historical FFPE tissue blocks may affect DNA quality and subsequent PCR amplification efficiency. Nevertheless, our

findings align with previously published literature and support the role of TCR clonality analysis as a valuable adjunctive diagnostic tool.

Overall, our results confirm that PCR-based detection of TCRγ gene rearrangements contributes significantly to the diagnostic evaluation of suspected early MF. However, the technique should not be used as a standalone diagnostic test. Instead, it must remain part of a comprehensive diagnostic approach integrating clinical presentation, histopathological examination, and immunophenotypic analysis.

5. Conclusion

The detection of TCRγ gene rearrangements via PCR significantly enhances the diagnostic workflow for early-stage mycosis fungoides, particularly in cases where clinical, histopathological, and immunohistochemical data are discordant.

While this molecular approach provides essential evidence of T-cell clonality, its sensitivity remains highly contingent upon factors such as tumor burden, tissue heterogeneity, and DNA preservation. Therefore, TCRγ molecular analysis should not be viewed as a definitive standalone test.

Instead, it must be integrated into a multidisciplinary diagnostic framework that correlates molecular data with clinical and morphological findings. This comprehensive strategy ultimately improves diagnostic precision and supports timely therapeutic decision-making in early-stage disease.

Author contributions

YS, Dr NZ, dr FS and SB contributed to literature review and analysis. MW contributed to data collection and design study. The first draft of the manuscript was written by HH and MW. MM. drafted the final manuscript. All authors critically revised the manuscript and approved the final version.

Consent for Patient Participation

Not applicable.

Consent for Publication

Not applicable.

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Conflict of Interest Disclosures

The authors declare that they have no conflict of interest

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